

*What Is Claimed Is:*

5 1. A method of treating amyloidosis in a subject, said method comprising administering to said subject an effective amount of (a) a metal chelator selected from the group consisting of: bathocuproine, bathophenanthroline, penicillamine, TETA, TPEN or hydrophobic derivatives thereof; and (b) one or more pharmaceutically acceptable carriers or diluents; for a time and under conditions to bring about said treatment; and

wherein said chelator reduces, inhibits or otherwise interferes with A $\beta$ -mediated production of radical oxygen species.

10 2. The method of claim 1 further comprising administering to the subject an effective amount of a compound selected from the group consisting of: rifampicin, disulfiram, and indomethacin, or a pharmaceutically acceptable salt thereof.

15 3. A method of treating amyloidosis in a subject, said method comprising administering to said subject a combination of (a) a metal chelator selected from the group consisting of: bathocuproine, bathophenanthroline, DTPA, EDTA, EGTA, penicillamine, TETA, and TPEN, or hydrophobic derivatives thereof; and (b) a supplement selected from the group consisting of: ammonium salt, calcium salt, magnesium salt, and sodium salt, for a time and under conditions to bring about said treatment; and

20 wherein said chelator reduces, inhibits or otherwise interferes with A $\beta$ -mediated production of radical oxygen species.

4. The method of claim 3 wherein the metal chelator is EGTA.

5. The method of claim 3 wherein the metal chelator is TPEN.

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6. The method of claim 3 wherein the supplement is magnesium salt.
7. The method of claim 3 further comprising administering to the subject an effective amount of a compound selected from the group consisting of: rifampicin, disulfiram, and indomethacin, or a pharmaceutically acceptable salt thereof.
8. A method of treating amyloidosis in a subject, said method comprising administering to said subject an effective amount of a salt of a metal chelator, wherein said chelator is selected from the group consisting of: bathocuproine, bathophenanthroline, DTPA, EDTA, EGTA, penicillamine, TETA, and TPEN, or hydrophobic derivatives thereof; wherein said salt of a metal chelator is selected from the group consisting of: ammonium, calcium, magnesium, and sodium; and wherein said salt of a metal chelator reduces, inhibits or otherwise interferes with A $\beta$ -mediated production of radical oxygen species.
9. The method according to claim 8 wherein the metal chelator is EGTA.
10. The method according to claim 8 wherein the metal chelator is TPEN.
11. The method according to claim 8 wherein the salt of a metal chelator is a magnesium salt.
12. The method according to claim 8 further comprising administering to said subject a compound selected from the group consisting of: rifampicin, disulfiram, and indomethacin, or a pharmaceutically acceptable salt thereof.

13. A method of treating amyloidosis in a subject, said method comprising administering to said subject an effective amount of a chelator specific for copper; wherein said chelator reduces, inhibits or otherwise interferes with A $\beta$ -mediated production of radical oxygen species.

14. The method of claim 13 wherein the chelator specific for copper is specific for the reduced form of copper.

15. The method of claim 14 wherein the chelator is bathocuproine or a hydrophobic derivative thereof.

16. A method of treating amyloidosis in a subject, said method comprising administering to said subject an effective amount of an alkalinizing agent, wherein said alkalinizing agent reduces, inhibits or otherwise interferes with A $\beta$ -mediated production of radical oxygen species.

17. The method of claim 16 wherein the alkalinizing agent is magnesium citrate.

18. The method of claim 16 wherein the alkalinizing agent is calcium citrate.

19. A method of treating amyloidosis in a subject, said method comprising administering to said subject an effective amount of (a) a metal chelator selected from the group consisting of: bathocuproine, bathophenanthroline, penacillamine, TETA, TPEN or hydrophobic derivatives thereof; and (b) one or more pharmaceutically acceptable carriers or diluents; for a time and under conditions to bring about said treatment; and wherein said chelator prevents formation of A $\beta$  amyloid, promotes, induces or otherwise facilitates resolubilization of A $\beta$  deposits, or both.

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20. The method of claim 19 further comprising administering to the subject an effective amount of a compound selected from the group consisting of: rifampicin, disulfiram, and indomethacin, or a pharmaceutically acceptable salt thereof.

21. A method of treating amyloidosis in a subject, said method comprising administering to said subject a combination of (a) a metal chelator selected from the following group: bathocuproine, bathophenanthroline, DTPA, EDTA, EGTA, penacillamine, TETA, and TPEN, or hydrophobic derivatives thereof; and (b) a supplement selected from the group consisting of: ammonium salt, calcium salt, magnesium salt, and sodium salt, for a time and under conditions to bring about said treatment; and

wherein said combination prevents formation of A $\beta$  amyloid, promotes, induces or otherwise facilitates resolubilization of A $\beta$  deposits, or both.

22. The method of claim 21 wherein the metal chelator is EGTA.

23. The method of claim 21 wherein the metal chelator is TPEN.

24. The method of claim 21 wherein the supplement is magnesium salt.

25. The method of claim 21 further comprising administering to the subject an effective amount of a compound selected from the group consisting of: rifampicin, disulfiram, and indomethacin, or a pharmaceutically acceptable salt thereof.

26. A method of treating amyloidosis in a subject, said method comprising administering to said subject an effective amount of a salt of a metal chelator, wherein said chelator is selected from the group consisting of:

bathocuproine, bathophenanthroline, DTPA, EDTA, EGTA, penacillamine, TETA, and TPEN, or hydrophobic derivatives thereof; wherein said salt of a metal chelator is selected from the group consisting of: ammonium, calcium, magnesium, and sodium; and wherein said salt of a metal chelator prevents formation of A $\beta$  amyloid, promotes, induces or otherwise facilitates resolubilization of A $\beta$  deposits, or both.

27. The method of claim 26 wherein the metal chelator is EGTA.

28. The method of claim 26 wherein the metal chelator is TPEN.

29. The method of claim 26 wherein the salt of a metal chelator is a magnesium salt.

30. The method of claim 26 further comprising administering to said subject a compound selected from the group consisting of: rifampicin, disulfiram, and indomethacin, or a pharmaceutically acceptable salt thereof.

31. A method of treating amyloidosis in a subject, said method comprising administering to said subject an effective amount of a chelator specific for copper; wherein said chelator prevents formation of A $\beta$  amyloid, promotes, induces or otherwise facilitates resolubilization of A $\beta$  deposits, or both.

32. The method of claim 31 wherein the chelator specific for copper is specific for the reduced form of copper.

33. The method of claim 31 wherein the chelator is bathocuproine or a hydrophobic derivative thereof.

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34. A method of treating amyloidosis in a subject, said method comprising administering to said subject an effective amount of an alkalinizing agent, wherein said alkalinizing agent prevents formation of A $\beta$  amyloid, promotes, induces or otherwise facilitates resolubilization of A $\beta$  deposits, or both.

35. The method of claim 16 wherein the alkalinizing agent is magnesium citrate.

36. The method of claim 16 wherein the alkalinizing agent is calcium citrate.

37. A pharmaceutical composition for treatment of conditions caused by amyloidosis, A $\beta$ -mediated ROS formation, or both, comprising: (a) a metal chelator selected from the group consisting of: bathocuproine, bathophenanthroline, penicillamine, TETA, and TPEN, or hydrophobic derivatives thereof; and (b) one or more pharmaceutically acceptable carriers or diluents.

38. The pharmaceutical composition of claim 37 further comprising a compound selected from the group consisting of: rifampicin, disulfiram, and indomethacin, or a pharmaceutically acceptable salt thereof.

39. A pharmaceutical composition for treatment of conditions caused by amyloidosis, A $\beta$ -mediated ROS formation, or both, comprising: (a) a metal chelator selected from the group consisting of: bathocuproine, bathophenanthroline, DTPA, EDTA, EGTA, penicillamine, TETA, and TPEN, or hydrophobic derivatives thereof; and (b) a supplement selected from the group consisting of: ammonium salt, calcium salt, magnesium salt, and sodium salt, together with one or more pharmaceutically acceptable carriers or diluents.

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40. The pharmaceutical composition of claim 39 wherein the metal chelator is EGTA.

41. The pharmaceutical composition of claim 39 wherein the metal chelator is TPEN.

42. The pharmaceutical composition of claim 39 wherein the supplement is a magnesium salt.

43. A pharmaceutical composition for treatment of conditions caused by amyloidosis, A $\beta$ -mediated ROS formation, or both, comprising a salt of a metal chelator selected from the group consisting of: bathocuproine, bathophenanthroline, DTPA, EDTA, EGTA, penicillamine, TETA, and TPEN, or hydrophobic derivatives thereof; and wherein said salt of a metal chelator is selected from the group consisting of ammonium, calcium, magnesium, and sodium, together with one or more pharmaceutically acceptable carriers or diluents.

44. The pharmaceutical composition of claim 43 wherein the metal chelator is EGTA.

45. The pharmaceutical composition of claim 43 wherein the metal chelator is TPEN.

46. The pharmaceutical composition of claim 43 wherein the salt of the chelator is a magnesium salt.

47. A pharmaceutical composition for treatment of conditions caused by amyloidosis, A $\beta$ -mediated ROS formation, or both, comprising a chelator

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specific for copper, with one or more pharmaceutically acceptable carriers or diluents.

48. The pharmaceutical composition of claim 47 wherein the chelator is specific for the reduced form of copper.

49. The pharmaceutical composition of claim 48 wherein the chelator specific for the reduced form of copper is bathocuproine.

50. A pharmaceutical composition for treatment of conditions caused by amyloidosis,  $A\beta$ -mediated ROS formation, or both, comprising an alkalinizing agent, with one or more pharmaceutically acceptable carriers or diluents.

51. The pharmaceutical composition of claim 50 wherein the alkalinizing agent is magnesium citrate.

52. The pharmaceutical composition of claim 50 wherein the alkalinizing agent is magnesium citrate.

53. A composition of matter comprising: (a) a metal chelator selected from the group consisting of: bathocuproine, bathophenanthroline, penicillamine, TETA, and TPEN, or hydrophobic derivatives thereof; and (b) a compound selected from the group consisting of: rifampicin, disulfiram, and indomethacin.

54. A composition of matter comprising: (a) a metal chelator selected from the group consisting of: bathocuproine, bathophenanthroline, DTPA, EDTA, EGTA, penicillamine, TETA, and TPEN, or hydrophobic derivatives thereof; and (b) a supplement selected from the group consisting of: ammonium salt, calcium salt, magnesium salt, and sodium salt.



55. The composition of claim 54 wherein the metal chelator is EGTA.
56. The composition of claim 54 wherein the metal chelator is TPEN.
57. The composition of claim 54 wherein the supplement is a magnesium salt.
58. A method for determining which metal chelators used in the treatment of amyloidosis, should be supplemented with ammonium, calcium, magnesium, or sodium salts, comprising:
- (a) contacting A $\beta$  aggregates with solutions containing a range of concentrations of said metal chelators;
  - (b) preparing a dilution curve from data obtained in step (a);
  - (c) selecting chelators which solubilize less A $\beta$  aggregates at higher concentrations than at lower or intermediate concentrations;
  - (d) contacting A $\beta$  aggregates with chelators selected in step (c), in the presence of an ammonium, calcium, magnesium or sodium salt; and
  - (e) determining if resolubilization is increased in the presence of said salt; thereby determining whether a metal chelator used in the treatment of amyloidosis should be supplemented with ammonium, calcium, magnesium, or sodium salts.
59. A method for the identification of an agent to be used in the treatment of AD, wherein said agent is capable of altering the production of Cu(I) by A $\beta$ , said method comprising:
- (a) adding Cu(II) to a first A $\beta$  sample;
  - (b) allowing said first sample to incubate for an amount of time sufficient to allow said first sample to generate Cu(I);
  - (c) adding Cu(II) to a second A $\beta$  sample, said second sample additionally comprising a candidate pharmacological agent;

(d) allowing said second sample to incubate for the same amount of time as said first sample;

(e) determining the amount of Cu(I) produced by said first sample and said second sample; and

(f) comparing the amount of Cu(I) produced by said first sample to the amount of Cu(I) produced by said second sample; whereby a difference in the amount of Cu(I) produced by said first sample as compared to said second sample indicates that said candidate pharmacological agent has altered the production of Cu(I) by A $\beta$ .

60. The method of claim 59, wherein the amount of Cu(I) present in said first and said second sample is determined by

(a) adding a complexing agent to said first and said second sample, wherein said complexing agent is capable of combining with Cu(I) to form a complex compound, wherein said complex compound has an optimal visible absorption wavelength;

(b) measuring the absorbancy of said first and said second sample; and

(c) calculating the concentration of Cu(I) in said first and said second sample using the absorbancy obtained in step (b).

61. The method of claim 60, wherein said complexing agent is bathocuproinedisulfonic anion.

62. The method of claim 60 or claim 61, wherein said method is performed in a microtiter plate, and the absorbancy measurement is performed by a plate reader.

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63. The method of claim 62, wherein two or more different test candidate agents are simultaneously evaluated for an ability to alter the production of Cu(I) by A $\beta$ .

64. The method of claim 59, wherein said first A $\beta$  sample of step 1(a) and said second A $\beta$  sample of step 1(c) is a biological sample.

65. The method of claim 64, wherein said biological sample is CSF.

66. A method for the identification of an agent to be used in the treatment of AD, wherein said agent is capable of altering the production of Fe(II) by A $\beta$ , said method comprising:

- (a) adding Fe(III) to a first A $\beta$  sample;
  - (b) allowing said first sample to incubate for an amount of time sufficient to allow said first sample to generate Fe(II);
  - (c) adding Fe(III) to a second A $\beta$  sample, said second sample additionally comprising a candidate pharmacological agent;
  - (d) allowing said second sample to incubate for the same amount of time as said first sample;
  - (e) determining the amount of Fe(II) produced by said first sample and said second sample; and
  - (f) comparing the amount of Fe(II) present in said first sample to the amount of Fe(II) present in said second sample;
- whereby a difference in the amount of Fe(II) present in said first sample as compared to said second sample indicates that said candidate pharmacological agent has altered the production of Fe(II) by A $\beta$ .

67. The method of claim 66, wherein the amount of Fe(II) present in said first and said second sample is determined by

(a) adding a complexing agent to said first and said second sample, wherein said complexing agent is capable of combining with Fe(II) to form a complex compound, wherein said complex compound has an optimal visible absorption wavelength;

(b) measuring the absorbancy of said first and said second sample; and

(c) calculating the concentration of Fe(II) in said first and said second sample using the absorbancy obtained in step (b).

68. The method of claim 67, wherein said complexing agent is bathophenanthrolinedisulfonic (BP) anion.

69. The method of claim 67 or claim 68, wherein said method is performed in a microtiter plate, and the absorbancy measurement is performed by a plate reader.

70. The method of claim 69, wherein two or more different test candidate agents are simultaneously evaluated for an ability to alter the production of Fe(II) by A $\beta$ .

71. The method of claim 66, wherein said first A $\beta$  sample of step 1(a) and said second A $\beta$  sample of step 1(c) is a biological sample.

72. The method of claim 71, wherein said biological sample is CSF.

73. A method for the identification of an agent to be used in the treatment of AD, wherein said agent is capable of altering the production of H<sub>2</sub>O<sub>2</sub> by A $\beta$ , said method comprising:

(a) adding Cu(II) or Fe(III) to a first A $\beta$  sample;

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(b) allowing said first sample to incubate for an amount of time sufficient to allow said first sample to generate  $H_2O_2$ ;

(c) adding Cu(II) or Fe(III) to a second A $\beta$  sample, said second sample additionally comprising a candidate pharmacological agent;

(d) allowing said second sample to incubate for the same amount of time as said first sample;

(e) determining the amount of  $H_2O_2$  produced by said first sample and said second sample; and

(f) comparing the amount of  $H_2O_2$  present in said first sample to the amount of  $H_2O_2$  present in said second sample; whereby a difference in the amount of  $H_2O_2$  present in said first sample as compared to said second sample indicates that said candidate pharmacological agent has altered the production of  $H_2O_2$  by A $\beta$ .

74. The method of claim 73, wherein the A $\beta$  samples of steps (a) and step (b) are a biological fluid.

75. The method of claim 74, wherein said biological fluid is CSF.

76. The method of claim 73, wherein the determination of step (e) of the amount of  $H_2O_2$  present in said first and said second sample is determined by

(a) adding catalase to a first aliquot of said first sample obtained in step (a) of claim 1 in an amount sufficient to break down all of the  $H_2O_2$  generated by said sample;

(b) adding TCEP, in an amount sufficient to capture all of the  $H_2O_2$  generated by said samples, to

(i) said first aliquot

(ii) a second aliquot of said first sample obtained in step (a) of claim 1; and

(iii) said second sample obtained in step (b) of claim 1;

- 135 -

(c) incubating the samples obtained in step (b) for an amount of time sufficient to allow the TCEP to capture all of the  $H_2O_2$ ;

(d) adding DTNB to said samples obtained in step (c);

(e) incubating said samples obtained in step (d) for an amount of time sufficient to generate TMB;

(f) measuring the absorbancy at 412 nm of said samples obtained in step (e); and

(g) calculating the concentration of  $H_2O_2$  in said first and said second sample using the absorbancies obtained in step (f).

77. The method of claim 76, wherein said method is performed in a microtiter plate, and the absorbancy measurement is performed by a plate reader.

78. The method of claim 77, wherein two or more different test candidate agents are simultaneously evaluated for an ability to alter the production of  $H_2O_2$  by  $A\beta$ .

79. A method for the identification of an agent to be used in the treatment of AD, wherein said agent is capable of interfering with the interaction of  $O_2$  and  $A\beta$  to produce  $O_2^-$ , without interfering with the SOD-like activity of  $A\beta$ , said method comprising:

(a) identifying an agent capable of decreasing the production of  $O_2^-$  by  $A\beta$ ; and

(b) determining the ability of said agent to alter the SOD-like activity of  $A\beta$ .

80. The method of claim 79, wherein the determination of the ability of said agent to alter the SOD-like activity of  $A\beta$  is made by determining whether  $A\beta$  is capable of catalytically producing  $Cu(I)$ ,  $Fe(II)$  or  $H_2O_2$ .

81. A method for the identification of an agent to be used in the treatment of AD, wherein said agent is capable of reducing the toxicity of A $\beta$ , said method comprising:

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- (a) adding A $\beta$  to a first cell culture;
  - (b) adding A $\beta$  to a second cell culture, said second cell culture additionally containing a candidate pharmacological agent;
  - (c) determining the level of neurotoxicity of A $\beta$  in said first and said second samples; and
  - (d) comparing the level of neurotoxicity of A $\beta$  in said first and said second samples,

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whereby a lower neurotoxicity level in said second sample as compared to said first sample indicates that said candidate pharmacological agent has reduced the neurotoxicity of A $\beta$ , and is thereby capable of being used to treat AD.

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82. The method of claim 81, wherein the neurotoxicity of A $\beta$  is determined by using an MTT assay.

83. The method of claim 81, wherein the neurotoxicity of A $\beta$  is determined by using an LDH release assay.

84. The method of claim 81, wherein the neurotoxicity of A $\beta$  is determined by using a Live/Dead assay.

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85. The method of claim 81, wherein said cells are rat cancer cells.

86. The method of claim 81, wherein said cells are rat primary frontal neuronal cells.

87. A kit for determining whether an agent is capable of altering the production of Cu(I) by A $\beta$  which comprises a carrier means being

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compartmentalized to receive in close confinement therein one or more container means wherein

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- (a) the first container means contains a peptide comprising A $\beta$  peptide;
  - (b) a second container means contains a Cu(II) salt; and
  - (c) a third container means contains BC anion.

88. The kit of claim 87, wherein said A $\beta$  peptide is present as a solution in an aqueous buffer or a physiological solution, at a concentration above about 10  $\mu$ M.

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89. A kit for determining whether an agent is capable of altering the production of Fe(II) by A $\beta$  which comprises a carrier means being compartmentalized to receive in close confinement therein one or more container means wherein

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- (a) the first container means contains a peptide comprising A $\beta$  peptide;
  - (b) a second container means contains an Fe(III) salt; and
  - (c) a third container means contains BP anion.

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90. The kit of claim 89, wherein said A $\beta$  peptide is present as a solution in an aqueous buffer or a physiological solution, at a concentration above about 10  $\mu$ M.

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91. A kit for determining whether an agent is capable of altering the production of H<sub>2</sub>O<sub>2</sub> by A $\beta$  which comprises a carrier means being compartmentalized to receive in close confinement therein one or more container means wherein

- (a) the first container means contains a peptide comprising A $\beta$  peptide;

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- (b) a second container means contains a Cu(II) salt;
- (c) a third container means contains TCEP; and
- (d) a fourth container means contains DTNB.

92. The kit of claim 91, wherein said A $\beta$  peptide is present as a solution in an aqueous buffer or a physiological solution, at a concentration above about 10  $\mu$ M.

93. A method for the identification of an agent to be used in the treatment of AD, wherein said agent is capable of inhibiting redox-reactive metal-mediated crosslinking of A $\beta$ , said method comprising:

- (a) adding a redox-reactive metal to a first A $\beta$  sample;
  - (b) allowing said first sample to incubate for an amount of time sufficient to allow A $\beta$  crosslinking;
  - (c) adding said redox-reactive metal to a second A $\beta$  sample, said second sample additionally comprising a candidate pharmacological agent;
  - (d) allowing said second sample to incubate for the same amount of time as said first sample;
  - (e) removing an aliquot from each of said first and said second sample; and
  - (f) determining presence or absence of crosslinking in said first and second samples,
- whereby an absence of A $\beta$  crosslinking in said second sample as compared to said first sample indicates that said candidate pharmacological agent has inhibited A $\beta$  crosslinking.

94. The method of claim 93, wherein at step (f), a western blot analysis is performed to determine the presence or absence of crosslinking in the first and the second sample.